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CONTRACTING ORGANIZATION: University of Kentucky Research Foundation
Lexington, Kentucky 40506-0057

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Delwood C. Collins, Ph.D.

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University of Kentucky Research Foundation
Lexington, Kentucky 40506-0057

E-mail: dccoll1@email.uky.edu

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Table of Contents

INTRODUCTION:

We have synthesized and characterized a unique group of diphenylureas that are much more potent than suramin or its sulfonated analogues as inhibitors of cell growth, migration and matrix metalloproteinase activity (MMP-2 and MMP-9) in human microvascular endothelial and human prostate cancer cell lines (PC3, LNCaP.FGC and DU145) in vitro. Toxicity studies in mice indicate no significant toxicity by the diphenylureas and a half-life 10-fold less than suramin. However, the diphenylureas do not bind the heparin-like growth factors. These results suggest that the diphenylureas could be potent inhibitors of prostate cancer in vivo that could reduce morbidity in man.

The experiments described in this proposal were the first in vivo test of the ability of a unique group of diphenylureas to inhibit the growth and metastasis in human androgen dependent (LNCaP) and androgen independent (PC3) prostate tumor xenografts in nude mice. We proposed that the diphenylureas were potent inhibitors of tumorigenesis, angiogenesis and metastasis in human prostate cancer growth. Furthermore, the antineoplastic activity of the diphenylureas was thought not to have its effect by inhibition of growth factors binding as has been demonstrated for suramin. These hypotheses were tested by the following questions:

Question #1. Are selected diphenylureas that inhibited growth of human prostate cancer cell lines in vitro able to inhibit the growth of human androgen dependent (LNCaP) and androgen independent (PC3) prostate cancer tumors in the athymic nude mouse model?

Question #2. Do the selected diphenylureas inhibit tumor angiogenesis in prostate cancer in vivo?

Question #3. Do the selected diphenylureas inhibit the metastatic ability of prostate cancer in vivo?

Positive results from these experiments could lead to translational research and clinical testing of these diphenylureas for prostate cancer therapy in man during Phase 2 of this proposal.

BODY

Experiment #1: Establish Growth Characteristics of PC3 Cells in Nude Mice in vivo.

Twelve nude mice were injected subcutaneously (s.c.) in each flank with media containing PC3 cells obtained from ATCC. Beginning five days later, six mice were injected intraperitoneally (i.p.) with saline containing 140 μmoles of NF681/kg body weight on alternate days for a total of five days. The remaining six mice were injected with saline only on the same regimen. Animals were observed for the next 90 days for visible tumors. Both of the injection sites were checked and no tumors were found. The remaining animals were euthanitized and the injection sites were carefully inspected for abnormal areas that might have been minute tumors. Only one animal showed a suspicious area. H & E-stained sections of the excised tissue were examined by the clinical pathologist and no tumor tissue was identified.

Results: No tumors were formed in any of the 12 mice injected with PC3 cells obtained from ATCC after 92 days of observation. These results were unexpected and led us to consult with other groups who have used this nude mouse model.

Experiment #2: Effect of Matrigel on Tumor Growth in Nude Mice in vivo.

Nine mice were injected with PC3 cells suspended in Matrigel at three sites on each animal (nape of neck and each flank). After nine days, tumors had formed at each injection site in eight of the nine animals while the remaining animal had tumors on two of three sites. The animals were then separated into control and treatment groups so that each group contained comparable numbers/size of tumors. Animals were injected i.p. with either 140 μmoles/kg body weight saline containing NF681 or saline only for six consecutive days before alternating days of injection for the remaining 22 days of the treatment period (11 additional injections). Animals were sacrificed on day 29 and tumors were collected.

Results: Nude mice injected with PC3 cells suspended in Matrigel grew tumors more rapidly than animals injected with media containing PC3 cells in Experiment #1. Tumors in one animal appeared to spontaneously regress during the last six days of the treatment period. The results from this animal were excluded from the control mean. The mean weight of the tumors (gm) and the tumor volume (mm^2) were not significantly different in the control animals compared to those animals treated with 140 μmoles/kg body weight after 22 days of treatment.

Experiment #3: Growth Characteristics of PC3 Cells in Nude Mice in vivo.

Eighteen athymic nude mice were injected s.c. in each flank with media containing PC3 cells. Beginning ten days after the injections, the animals were divided randomly into three treatment groups: control, NF681 and NF050. Each animal was treated daily with 140 μmoles/kg body weight for ten consecutive days. Subsequently, the injection regimen was changed to daily injections for five days, followed by no injections for two days for the remaining 28 days of the experiment.

Results: The mean tumor weight ± SD was 1,670 ± 467 mg for NF681 treated animals compared to 1,818 ± 1,112. The mean tumor volumes were 1,429 ± 682 cubic mm for NF681 treated mice compared to 1,020 ± 531. Tumor weights and tumor volumes did not show a significant reduction in PC3 tumor size in animals treated with 140 μmoles/kg body weight of NF681 or NF050 for 28 days. This was attributed to the slow and variable growth of the tumors in the nude mouse xenograft model and the small number of mice in each group (4 mice per group).

It was noted during autopsy of mice treated with NF681 that a white material was deposited in the peritoneal cavity. This white material was collected and subjected to HPLC chromatography as described by Kassack and Nickel (1996). The white material appeared to be NF681, based on its retention times on HPLC. It appeared that NF681 precipitated after injection into the peritoneal cavity. Further experiments showed that the pH of NF681 and NF050 in water was 8.5 and 9.0, respectively. Thus, they would likely precipitate in the slightly acidic pH of the peritoneal cavity.

Experiment #4: Development of a PC3 Tumor Line Derived from a Nude Mouse PC-3 Tumor Xenograft.

Because of the difficulty encountered in establishing a PC3 nude mouse xenograft that grew at a reasonable and consistent rate, we requested a modification of the work plan and a one year extension of the grant period without additional funds. We also proposed the following changes in the statement of work.

1. The growth of PC3 cells obtained from ATCC was very slow, with many controls not showing a tumor after 50 days. However, the PC3 cells obtained from ATCC grown in a nude mouse xenograft showed a much higher growth rate both in vitro and in subsequent in vivo xenograft experiments. These cells (designated PC3-T cells) were used in PC3 xenografts in this experiment. On day 0, both flanks of the nude mouse were injected with 10^6 in 0.05 ml culture medium (Day 0).

2. Evidence described in Experiment #3 indicated that the solubility of NF681 and NF050 was poor after intraperitoneal injection as indicated by the large amount of NF681 and NF050 identified in the peritoneum. This suggested that the blood levels of NF681 and NF050 were inadequate to initiate an effect. In order to overcome this problem, NF681 was injected s.c. in the flank of the mouse adjacent to the area where the tumor cells were implanted, beginning one day after implantation of the PC3-T cell. This alteration in the protocol allowed the local levels of NF681 near the tumor to be adequate to initiate an effect on the growth of PC3 cells in vivo. This approach has been used by other investigators to assess the effect of poorly soluble compounds on cancer growth and angiogenesis.

3. Preliminary experiments were carried out on four mice in each group injected s.c. with 2 mg, 1 mg or 500 μg of NF68 in 0.05 ml of physiological saline (PBS) every other day. After one week, obvious tissue damage was observed at the 2 mg and 1 mg dose, but not at the 500 μg dose. The higher doses were stopped and the experiment was continued with subcutaneous injections of 500 μg every other day treatment for 3, 6 or 9 weeks. Controls were treated with 0.05 ml of PBS.

4. Because of the variability encountered in the growth of PC3 tumors in experiments 1-3, the number of animals in each test group was increased from 10 to 20 animals in the PC3-T xenograft model.

5. Task 2 studies on the androgen-dependent human prostate cancer (LNCaP) in nude mice xenografts were excluded because in vitro experiments in our laboratory suggested that the LNCaP prostate cancer cells were not inhibited as effectively by NF681 or NF050.

Results: The mean weight of the mice in the various treatment groups was collected at the completion of their study protocol. This information is shown in Table 1 in Appendix 1. There was no significant difference in the weight of the animals injected with NF681 and the controls at 3, 6 or 9 weeks. These results suggest that there was no significant effect of the NF681 injections on the body weight of the mice.

The tumors in the flanks of the mice were removed at autopsy and carefully dissected from the surrounding tissue. Tissues collected after 3, 6 and 9 weeks of treatment with NF681 were compared to the untreated controls (See Table 2 in Appendix 1). The mean weights of the tumors isolated from the flanks of the animals showed significant growth over the 9 weeks in both control and NF681 treated animals. After 3 weeks, the mean weights of the tumors were 79 and 67 mg, respectively, in NF681 treated and control animals. The mean weight of the tumors grew approximately 3-fold by 6 weeks and tripled again between 6 and 9 weeks.

The higher growth rate shown in the results in Table 2 clearly indicates that the PC3-T prostate cancer xenograft preparation grew at a much higher rate than the PC3 xenograft initially used in this grant. However, there was still significant variation in the growth rate of the tumors in different animals, leading to large standard deviations in the various groups. Furthermore, we were not able to demonstrate a significant difference in the tumor size of the NF681 treated animals compared to the untreated controls after 3, 6 or 9 weeks of treatment. These results do not support the hypothesis that NF681 inhibits the growth of PC3-T cells.

Experiment #5: Determine the Effect of NF681 Treatment on Tumor Proliferation.

If NF681 has an inhibitory effect on the cytokinetics of tumor growth as found in an in vitro preparation, we expected to see a significant reduction in the bromodeoxyuridine (BrdU) labeling in tumors of mice treated with NF681. This experiment was carried out on all of the animals described in experiment #4. BrdU labeling reagent (ZYMED Laboratories Inc., San Francisco, CA) was injected intraperitoneally (2 mg in 0.2 ml) into the mice two hours prior to sacrifice. BrdU, a thymidine analogue, is incorporated into proliferating cells. Tumor proliferation is a good measure of the rate of growth (cells in S-phase) and the effect of various compounds on tumor growth. Labeling of tumor cells with BrdU correlates highly with [^{3}H]thymidine, the gold standard for measuring tumor S-phase (Weidner et al, 1993).

Histology: Specimens for histological analysis were fixed in formalin and saline and processed to paraffin blocks. Sections were cut (5 μm). One section was stained with hematoxylin and the adjacent section was immunostained for BrdU using a monoclonal anti-BrdU antibody and detected with a sensitive streptatavidin-biotin staining system (Fukuda et al, 1990).

BrdU stained sections were quantitated by visual assessment of the whole specimen at 20x on a Nikon E800 microscope with a digital camera to select the areas of highest labeling in the tumor, usually near the edge of the tumor. In most cases, three areas of highest labeling of BrdU were selected and quantitated at 40x using the Metamorph Imaging Analysis software. Results were reported as the mean Labeling Index (LI) ± SD (i.e., the % staining for BrdU) for each animal.

Results: The mean BrdU Labeling Index for mice injected with 500 μg NF681 or untreated controls is shown in Table 3 of Appendix 1. There was no significant difference in the BrdU Labeling Index between the NF681 treated and the control animals. In addition, there was no effect of the time of treatment (i.e., 3, 6 and 9 weeks) on the Labeling Index. These results

clearly suggest that there was no significant effect of NF681 on the rate of proliferation of the PC3-T tumor xenograft under the conditions of this study. However, it should be noted that we have had considerable difficulty in delivering NF681 to the PC3-T xenograft.

Experiment #6: Quantitation of Microvessel Density in Prostate Tumor (PC3-T) Xenograft.

If NF681 has an inhibitory effect on the endothelial cell growth and migration as found in an in vitro preparation of endothelial cells, we expected to see a significant reduction in the BrdU labeling in tumors of mice treated with NF681. This experiment was carried out on all of the animals described in experiment #4. Tissues for microvessel density counting were processed, embedded in paraffin and sectioned onto slides. None of the Factor VIII, CD31 or CD34 human antibodies showed adequate cross-reactivity for the mouse endothelial cells in these xenografts. After considerable work, our laboratory has developed a murine procedure for Factor VIII in these tissues using a polyclonal biotenylated rabbit anti-human Factor VIII (DAKO Code # A0082, DAKO Corporation, Carpenteria, CA) that has a 30% cross-reactivity with murine Factor VIII (Technical Service Bulletin, DAKO).

Histology: Factor VIII was stained in tissues using a modification of a procedure described by Hunyady et al. (1996). Sections (5 μm) of each tumor were de-waxed and subjected to target retrieval using methods described above for BrdU immunohistochemistry. After the TBS washes, each section was blocked for endogenous biotin activity using the DAKO® Biotin Blocking System kit (DAKO Corporation, Carpenteria, CA). After the biotin-block, each section was blocked for 20 minutes against non-specific protein binding using the normal goat serum supplied with the Vectastain® ABC Alkaline Phosphatase Rabbit IgG kit (VECTOR Laboratories, Inc., Burlingame, CA). Each tissue section was then incubated with a 1:75 dilution of rabbit anti-human Factor VIII (DAKO Corporation, Carpenteria, CA) that has about 30% cross-reactivity for the murine Factor VIII-related protein. After washing in TBST (Tris-buffered saline with 0.01% Tween 20), the biotinylated secondary antibody (goat anti-rabbit IgG) supplied in the Vecstatin® ABC Alkaline Phosphatase Rabbit IgG kit (VECTOR Laboratories, Inc., Burlingame, CA) was diluted according to kit directions and applied to each tissue section for 30 minutes. After 3 x 2- minutes TBST rinses, each section was incubated for 30 minutes with the Vectastain ABC-AP enzyme reagent from the Vectastain® ABC Alkaline Phosphatase Rabbit IgG kit (VECTOR Laboratories, Inc., Burlingame, CA). After 3 x 2-minutes rinses with TBST, freshly prepared Vector Red Alkaline Phosphatase substrate reagent with levamisole (VECTOR Laboratories, Inc., Burlingame, CA) and the reagents from the VECTOR Alkaline Phosphatase Substrate kit 1 (VECTOR Laboratories, Inc., Burlingame, CA) were incubated with the tissues for 15-30 minutes in the dark to control color development. After a brief rinsing in tap water to stop color development, each slide was counterstained in Harris' hematoxylin solution for 4-5 minutes. A Nikon E800 microscope connected to a digital camera was used to identify areas of high density staining ("hot spots") of Factor VIII at multiple sites within each tumor. MetaMorph Image Analysis software was used to quantitate Factor VIII within each "hot spot".

Results: The results of the experiment to test the inhibitory effect of NF681 on the growth of PC3-T prostate cancer xenografts in nude mice are shown in Table 4 in Appendix 1. Tumors were stained for Factor VIII to measure the presence of murine endothelial cells. As expected,

most of the Factor VIII staining occurred near the edge of the tumor. However, there was wide variation between the individual "hot spots" of Factor VIII staining. This information correlates with the higher levels of BrdU labeling that also occurred near the edge of the tumor and suggests that the major growth of the PC3-T xenograft occurs in the same region. Contrary to our finding that NF681 was an effective inhibitor of angiogenesis in vitro in human endothelial cells, we were unable to demonstrate a significant effect on angiogenesis in the murine endothelial cells of the PC3-T xenograft used in this experiment.

Experiment #7. Metalloproteinase (MMP) Activity in Prostate Carcinoma.

Several reports have shown a correlation between MMP activity and metastasis in prostate carcinoma. MMP activity was measured in media of prostate carcinoma cell lines in vitro and in prostate tumors from the mouse xenograft using a polyacrylamide gel electrophoresis zymograph method.

PC3 and DU145 cell cultures showed large amounts of MMP-9 activity but low MMP-2 activity in vitro. On the other hand, no MMP activity was identified in culture media from LNCaP cells. MMP-2 activity was enhanced by addition of epidermal growth factor (EGF) (40 μg/L) in DU145 but not in PC3 or LNCaP. When PC3 tumor cells obtained from the nude mouse xenograft were isolated and placed back in culture, the MMP activity was markedly different from the original PC3 cell line.

- Pro MMP-9 activity was not present in cells isolated from the xenograft (PC3-T cells).
- MMP-2 activity was sharply increased to a level similar to MMP-9 in PC3-T cells.
- NF681 and NF050 were potent inhibitors of MMP-2 and MMP-9 activity in PC3 cells in vitro but suramin had no effect on MMP activity.
- Incubation of PC3-conditioned media with NF681 or NF050 did not affect MMP activity.

These results suggest that MMP activity may be an important factor in prostate carcinoma cells. This activity is inhibited by the phosphonic acid diphenylureas (NF681 and NF050) but not by suramin. Furthermore, the phosphonic acid diphenylureas do not directly inhibit MMP activity, suggesting that they may function by inhibition of the synthesis or secretion of MMPs. This is a very important and novel activity that has not been previously reported.

Using tumor samples from Experiment 4, we measured the MMP-2 and MMP-9 activity of the tumors using a standard zymograph procedure as previously described. The results were similar to those described above. However, we were unable to demonstrate inhibition of the MMP-9 or MMP-2 activity in PC3-T xenograft cells taken from animals treated with 500 μg NF681 every other day for 3, 6 or 9 weeks.

Key Research Accomplishments

1. We were not able to demonstrate inhibition of PC3-T growth in the nude mouse xenograft by the diphenylurea, NF681, a compound shown to be much more effective than suramin in its cytotoxic effect in vitro. This was demonstrated by the inability of NF681 to inhibit tumor growth (experiment #4) and proliferation (BrdU incorporation) (experiment #5).

2. We were not able to demonstrate inhibition of angiogenesis by NF681, the most potent diphenylurea that inhibits human endothelial cell growth in vitro. This was demonstrated by the inability of NF681 to inhibit endothelial cell growth (Factor VIII staining) at the growth edge of the PC3-T mouse xenograft

3. We demonstrated that the phosphonic acid diphenylureas are potent inhibitors of MMP activity in prostate carcinoma cells in vitro. The results suggest that this inhibition is not a direct effect on MMP activity, but may be inhibition of MMP synthesis or secretion. This is the first compound reported to show this activity. In the PC3-T mouse xenograft, we were able to demonstrate a change in MMP activity with a significant increase in MMP-2 activity equal to MMP-9 activity. However, we were unable to demonstrate that treatment of mice with 500 μg NF681 every other day could inhibit NF681 activity as described in our laboratory in vitro in PC3 prostate tumor cells.

Reportable Outcomes:

1. **Abstracts and Presentations:**

Gagliardi, A.R.T., P. Nickel and D.C. Collins. Inhibition of Prostate Cancer Cell Growth by Diphenylureas, A Unique Group of Antiangiogenic Compounds. American Association for Cancer Research, "Novel Approaches to Organ Site-Specific Therapies" Minisymposium, 1998.

Gagliardi, A.R., R.K. Munn, P. Nickel and D.C. Collins. Inhibition of Prostate Cancer Cell Growth by a Unique Group of Antiangiogenic Compounds. Fourth International Symposium on Predictive Oncology and Therapy, Nice, France, 1998.

Collins, D.C., A.R.T. Gagliardi and P. Nickel. Inhibition of Prostate Cancer Growth, Metalloproteinase (MMP) Activity and Urokinase-Plasminogen Activator (uPA) by Phosphonic Acid Diphenylureas. American Association for Cancer Research Symposium "New Research Approaches in the Prevention and Cure of Prostate Cancer", abstract, 1998.

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Gagliardi A., D.C. Collins and P. Nickel. Phosphonic Acid Diphenylureas, A Unique Group of Potent Antiangiogenic and Antitumorigenic Compounds. Fifth Biannual International Conference, "Angiogenesis: From the Molecular to Integrative Pharmacology", Crete, Greece, abstract, 1999.

Gagliardi, A., R.K. Munn and D.C. Collins. The Effect of Tamoxifen on the Angiogenesis Cascade. Fifth Biannual International Conference, "Angiogenesis: From the Molecular to Integrative Pharmacology", Crete, Greece, abstract, 1999.

Gagliardi, A.R.T., M. Bittencourt, P. Nickel, D.C. Collins. Inhibition of Renal Cell Carcinoma Growth and Metalloproteinase Activity by NF681, A Phosphonic Acid Diphenylurea. AACR-NCI-EORTC International Conference "Molecular Targets and Cancer Therapeutics", Washington, abstract, 1999.

Gagliardi, A.R.T., M. Bittencourt, P. Nickel, D.C. Collins. The Effect of a Phosphonic Acid Diphenylurea, NF681, on Renal Carcinoma Cell Growth and Metalloproteinase Activity. First International Kidney Cancer Symposium, Chicago, abstract, 1999.

Gagliardi, A.R.T., M. Bittencourt, P. Nickel, D.C. Collins. Inhibition of Renal Cell Carcinoma Growth and Metalloproteinase activity by NF681, a Phosphonic Acid Diphenylurea. American Association for Cancer Research, abstract, 2000.

2. Publications:

Gagliardi, A.R.T., M. Kassack, A. Kreimeyer, G. Muller, P. Nickel and D.C. Collins. Antiangiogenic and Antiproliferative Activity of Suramin Analogues. Cancer Chemotherapy and Pharmacology, 41:117-124, 1998.

Gagliardi, A.R., D.C. Collins and P. Nickel. Diphenylureas are Potent Inhibitors of Angiogenesis. Proceedings of the 17th UICC International Cancer Congress, pp. 221-225, 1998.

Gagliardi, A.R.T., P. Nickel and D.C. Collins. Inhibition of Prostate Cancer Cell Growth by Diphenylureas, A Unique Group of Antiangiogenic Compounds. Proceedings of the 17th UICC International Cancer Congress, pp. 1109-1113, 1998.

Gagliardi, A.R., M.C. Bittencourt, R.K. Munn, P. Nickel and D.C. Collins. Inhibition of Renal Carcinoma Cell Growth by Diphenylureas, a Unique Group of Antiangiogenic Compounds. Proceedings of the 17th UICC International Cancer Congress, pp.1137-1140, 1998.

3. Patents:

Patent application entitled "Phosphonic Acid Diphenylureas. Methods for Synthesis, Antiangiogenic and Antitumorigenic Activity. Submitted with Antonio R.T. Gagliardi, M.D., Ph.D. and Peter Nickel, Ph.D. This patent was received in year 2000.

Conclusions:

The inability to demonstrate either of the in vitro effects above suggests that the diphenylurea compounds tested may not be good candidates for therapeutic application because of solubility and drug delivery problems to the tumor site. This was demonstrated by the poor solubility and the high pH (8.4) of NF681.

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Appendix 1

Tables 1-4

Award Number: DAMD17-98-1-8467

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PRINCIPAL INVESTIGATOR: Delwood C. Collins, Ph.D.

CONTRACTING ORGANIZATION:

204 Health Sciences Research Building
University of Kentucky Medical Center
Lexington, KY 40536-0305

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PREPARED FOR: U.S. Army Medical Research and Material Command
Fort Detrick, Maryland 21702-5012

Table 1. Mean body weight ± SD (gm) of male nude mice implanted with 2 x 10^6 prostate tumor cells (PC3-T) and injected with 500 µg NF681 in PBS or PBS alone (control) every other day for 3, 6 or 9 weeks.

Treatment Period (wk)	Treatment	Number Animals	Mean Body Wt (gm)	SD	Range
3	NF681	16	27.1	2.8	19.6 - 31.8
3	control	16	26.5	2.0	23.7 – 30.4
6	NF681	16	29.3	3.6	23.9 – 33.6
6	control	17	27.4	2.5	22.2 – 30.6
9	NF681	17	29.9	3.1	23.7 – 33.7
9	control	17	29.8	3.7	19.2 – 34.3

Table 2. Mean tumor weight ± SD (gm) in male nude mice implanted with 2 x 10^6 prostate tumor cells (PC3-T) and injected with 500 µg NF681 in PBS or PBS alone (control) every other day for 3, 6 or 9 weeks.

Treatment Period (wk)	Treatment	Number Animals	MeanTumor Wt (gm)	SD	Range
3	NF681	16	79	52	30 - 224
3	control	14	67	46	5 - 158
6	NF681	17	181	159	10 - 538
6	control	17	191	211	5 - 859
9	NF681	17	610	477	28 – 1,682
9	control	17	551	546	0 – 2,181

Table 3. Mean bromodeoxyuridine (BrdU) incoporation Labeling Index (i.e., % labeled) in male nude mice implanted with 2 x 10^6 prostate tumor cells (PC3-T) and injected with 500 µg NF681 in PBS or PBS alone (control) every other day for 3, 6 or 9 weeks.

Treatment Period (wk)	Treatment	Number Animals	Mean Labeling Index (%)	SD	Range
3	NF681	16	3.1	1.4	1.1 – 7.6
3	control	14	2.9	1.2	1.1 – 4.8
6	NF681	17	3.3	1.6	1.4 – 4.4
6	control	17	3.3	1.5	1.5 – 5.3
9	NF681	17	3.0	3.0	1.8 –5.0
9	control	14	3.2	1.3	1.2 – 6.4

Table 4: The percent of endothelial cells present as indicated by Factor VIII staining in male nude mice implanted with 2 x 10^6 prostate tumor cells (PC3-T) and injected with 500 µg NF681 in PBS or PBS alone (control) every other day for 3, 6 or 9 weeks.

Treatment Period (wk)	Treatment	Number Animals	Mean % Endothelial Cells	SD	Range
3	NF681	16	7.3	4.6	1.2 – 22.7
3	control	13	7.8	4.2	2.5 – 23.2
6	NF681	16	3.4	2.8	0.6 –11.2
6	control	17	4.5	2.8	2.4 – 10.1
9	NF681	17	6.2	2.4	1.5 –14.4
9	control	16	10.7	9.5	8.0 – 32.3

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US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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REPLY TO
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MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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